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HCV genotype 1 subtypes (1a and 1b): similarities and differences in clinical features and therapeutic outcome

Authors: A. Andriulli[1,], F. Morisco[2], A.M. Ippolito[1], V. Di Marco[3], M.R. Valvano[1], M. Angelico[4], G. Fattovich[5], Granata R[2], A. Smedile[6], M. Milella[7], M. Felder[8], G.B. Gaeta[9], P. Gatti[10], M. Fasano[11], G. Mazzella[12], T. Santantonio[11]

Affiliations:

[1] Division of Gastroenterology, Casa Sollievo Sofferenza Hospital, IRCCS, San Giovanni Rotondo, [2] Department of Clinical Medicine and Surgery, Federico II University of Naples, [3] Division of Gastroenterology, University of Palermo, [4] Division of Gastroenterology, Tor Vergata University, Roma, [5] Division of Gastroenterology, University of Verona, [6] Department of Gastroenterology and Hepatology, Azienda Ospedaliera Città della Salute e della Scienza, Torino, [7] Clinic of Infectious Diseases, University of Bari, [8] Division of Gastroenterology, Central Hospital, Bolzano, [9] Clinic of Infectious Diseases, University of Naples, [10] DIMO Medical Oncology, University of Bari, [11] Clinic of Infectious Diseases, University of Foggia, [12] Division of Gastroenterology, University of Bologna, ITALY.

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Address for correspondence: Angelo Andriulli, Division of Gastroenterology, Casa Sollievo Sofferenza Hospital, IRCCS, viale Cappuccini 1, San Giovanni Rotondo, ITALY

Phone: +39 0882 410263 Fax: + 39 0882 835411

Email: a.andriulli@operapadrepio.it

ABSTRACT

Aim: to evaluate in HCV-1 subtypes, 1a and 1b, similarities and differences in presenting clinical features, and the influence of baseline and predictors of response to peg-interferon and ribavirin (Peg/RIBA).

Patients and methods: 1233 naïve patients with HCV genotype-1 infection, 159 (13%) with subtype 1a, and 1074 (87%) with subtype 1b, were treated with Peg-IFN/RIBA at 12 Italian centres. Covariates included in the logistic model were age, gender, BMI, serum alanine aminotransferase (ALT), serum gamma-glutamiltranspeptidase (γGT), platelets counts, liver fibrosis, the occurrence of type 2 diabetes, baseline viremia, and IL28B genotype.

Results: At multivariate analysis, baseline characteristics differentiating patients with HCV-1a vs HCV-1b were young age, male gender, no F4 fibrosis, and no diabetes. SVR was achieved by 37% of patients with subtype 1b, and 45% of those with subtype 1a, a non significant 8% difference (p=0.069). In patients with subtype 1a, predictors of SVR were IL28B CC (OR=5.78, CI 1.98-16.83), RVR (OR=4.18, CI 1.66-10.55), female gender (OR=2.83, CI 1.83-6.78), and HCVRNA (OR=0.55, CI 0.32-0.96). In patients with subtype 1b the ranking of predictors was RVR (OR=6.49, CI 4.32-9.73), IL28B CC (OR=3.32, CI 2.15-4.58), γGT (OR=1.59, CI 0.14-2.22), HCVRNA (OR=0.61, CI 0.47-0.79), and age (OR=0.01, CI 0.02-0.42).

Conclusion: in Italy HCV-1 subtype 1a prevails in young, male patients with less advanced liver damage, findings which imply a more recent spreading of the infection with this viral strain. The two HCV-1 subtypes appear equally responsive to Peg-IFN/RIBA, with IL28B genotyping and monitoring of RVR mostly influencing the therapeutic response.

INTRODUCTION

In patients with chronic HCV infection, the efficacy of pegylated-interferon and Ribavirin (Peg-IFN and RIBA) is highly variable, with the HCV genotype exerting a major influence on the therapeutic response. Several clinical trials have concordantly reported that patients infected with HCV 1 and 4 strains have only a 40-50% likelihood of achieving a sustained viral response (SVR) following full- dose Peg-IFN and RIBA for 48 weeks (1-3).

HCV genotype 1 is generally considered as a homogeneous group, but it is composed of at least 60 subtypes with differences in nucleotide and amino acid sequences (4,5). Among subtypes, 1a and 1b strains are prominent. Major clinical trials evaluated treatment response among HCV-1a and 1b patients grouped together and, consequently, were unable to unveil a differential outcome of treatment between the two viral strains. Of available trials (7-14), only few studies were sufficiently powered to detect an eventual difference, with some reporting a lower efficacy of Peg-IFN and RIBA in HCV-subtype 1a, and other studies unraveling an opposite finding. In addition, the impact of HCV-1 subtypes on the on-treatment response milestones, such as the rapid viral response at treatment week 4 (RVR), has not been extensively investigated. Finally, no data on the distribution of the polymorphism of the IL28B gene and its influence of on-treatment viral response between the two HCV subtypes are yet available.

In an observational, real life study carried out in 12 Italian centers, we gathered information on a large group of patients with HCV-1 infection who were treated with Peg-IFN and RIBA (15,16). Aims of the present report were to evaluate baseline similarities and differences between the two HCV-1 subtypes in relation to patients' characteristics and severity of the hepatic disease, and to assess the influence of HCV-1 subtypes on on-treatment response. Finally, with the intention to reconcile discrepant results reported in different clinical trials on the relevance of subtyping for the

two HCV-1 strains on SVR, we pooled our data with those from other available studies in a meta-analysis.

PATIENTS AND METHODS

Study design and patients population

This is a sub-analysis of a multicentre study performed in a large, homogeneous cohort of naïve patients with HCV genotype-1. In the present study, we split the original database of 1233 patients according to HCV-1subtypes, 1a or 1b, with the intent to delineate presenting patients' features and their on- and off-treatment therapeutic response. Exclusion and inclusion criteria have been reported in previous studies on this same cohort of patients (14,15). Patients received combination therapy with Peg-IFN α-2a or α-2b plus RIBA (Rebetol capsules, Schering Corp., Kenilworth, NJ, USA; Copegus tablets, Roche, Basel, Switzerland), as recommended by international guidelines: Pegasys 180 mcg once weekly, subcutaneously; Peg-Intron 1.5 mcg/kg/week, subcutaneously; RIBA 1000 or 1200 mg/day orally depending on body weight. Treatment duration lasted 48 weeks for all patients who cleared the virus by treatment week 12; therapy was withdrawn at treatment week 24 for non-responsive patients or for the subset of those with RVR and good predictors at baseline (16-19).

Measurements

Serum HCV-RNA was quantified by Cobas Amplicor HCV Monitor Test, v 2.0 (Roche, Basel, Switzerland) with a lower detection of 50 IU/L. Baseline HCV-RNA level was expressed as $\log_{10}$ IU/ml. Virologic response was defined as undetectable HCV-RNA at treatment week 4 (RVR = rapid virological response), at week 12 (EVR = early virological response), at end of treatment (EOT), and at week 24 off-therapy (SVR). HCV was genotyped by Line Probe Assay (Inno-Lipa

HCV II; Innogenetics Zwijndrecht, Belgium. We genotyped the IL28B polymorphism rs12979860 using the TaqMan SNP kit (ABI TaqMan) and the ABI 7900HT sequence detection System (Applied Biosystems, Foster City, CA, USA).

Meta-analysis

With the intent to solve discrepancies in reported differences in treatment outcome between patients with HCV subtype 1a or 1b (6-13), we pooled available information in a meta-analysis. Trials were identified by searching MEDLINE from 2001 to 2013 by the following search keys: HCV genotypes AND ((therapy) OR (Peg-IFN) OR (RIBA)). Studies were excluded if reporting data on HIV- or HBV co-infected patients, on treatment experienced patients, treatment schedules consisting of conventional interferon as monotherapy or in combination with RIBA, or whenever data was not retrievable separately for HCV-1a or 1b. Manual searching included reading through reference lists of relevant papers to capture missing studies that met our inclusion criteria. Two independent reviewers (A.A., F.M.) scanned every studies identified by the search to determine eligibility. Extracted data included publication date, location of study, patient inclusion and exclusion criteria, mean age, male and female proportion, schedule, dose and duration of therapy. Primary outcome measure was rates of SVR for patients with HCV subtype 1a vs those with subtype 1B.

Statistical analysis

All categorical and continuous variables were described as absolute and percentage frequencies, and medians and interquartile ranges (IQR), respectively. Associations between categorical variables with the genotype 1a and 1b infection were evaluated by Pearson Chi-square test or Fisher's exact test. Continuous covariates were compared by Mann-Whitney test. Multivariate analysis was

performed by stepwise logistic regression model. Covariates included in the model were age, sex, BMI, serum alanine aminotransferase (ALT) levels, serum gamma-glutamiltranspeptidase (γGT) levels (abnormal vs normal), platelets counts, liver fibrosis stage (F4 vs <F4), the occurrence of type 2 diabetes, baseline viral load (IU/mL), IL28B genotype, type of Peg-IFN alpha (2a vs 2b), RVR, EVR, EOT, and SVR. Continuous variables that were not normally distributed were $\log_{10}$ transformed before analysis. A second model was built considering the same potential explanatory variables associated to SVR respectively in HCV subtype 1a and 1b. All analyses were performed using the SPS 13 software.

For the meta-analysis, data were analysed using the Comprehensive Meta-Analysis statistical software (version 1.0.25; Biostat, Englewood, NJ, USA). Pooled Odds Ratios (OR) along with corresponding 95% confidence intervals (CI) were calculated using a random effect model (20). Heterogeneity was investigated by using the I^2 statistic with significance set at $p < 0.05$ (21).

RESULTS

Baseline characteristics of patients associated with HCV subtype 1a or 1b

A total of 1233 chronic HCV-1 infected patients were recruited for the study. HCV subtype 1a was genotyped in 159 patients (13%), and subtype 1b in the remaining 1074 subjects (87%). The disposition of patients' characteristics in relation to viral subtypes is reported in Table 1. As to the median age, patients with HCV subtype 1a were 10 years younger (47 vs 57 years, $p<0.001$), and showed a gender association, being more often males (74% vs 56%; $p<0.001$). While serum ALT, γGT, and viral load levels did not differ, median platelets counts were higher in HCV subtype 1a, a finding in keeping with a difference in the severity of the hepatic damage: more subtype 1a patients had histological or instrumental features of less advanced (<F4) fibrosis of the liver (84% vs 70%; $p<0.001$). Despite equal BMI median values, more diabetic patients carried the subtype 1b, at a triple frequency of that encountered in subtype 1a (12% vs 4%; $p<0.003$). The distribution of

IL28B genotypes did not differ between the two subtypes. All parameters differentially and significantly distributed between the two HCV subtypes at univariate analysis were entered into a multivariate logistic model to assess the independent features differentiating the two subtypes. The main effects models showed that male gender (O.R. 2,40; C.I. 1,63-3,53), age (O.R. 0,95; C.I. 0,94-0,97), absence of F4 (O.R. 0,59; C.I. 0,37-0,95), and no type 2 diabetes (O.R. 0,45; C.I. 0,20-1,00) were independently associated with subtype 1a.

Virological outcome of HCV-1a vs HCV-1b following therapy with Peg-IFN and RIBA

In patients infected with the two HCV subtypes, on-treatment viral kinetics are shown in Figure 1. At week 4 of treatment, HCV-RNA was undetectable in 28 (28%) patients with subtype 1a, and in 244 (23%) patients with subtype 1b (p=0.168). Similarly, EVR and EOT rates were not dissimilar between carriers of the HCV-1a or -1b subtype. A total of 470 (38%) patients achieved a SVR, 71 of 159 (45%) with subtype 1a, and 399 of 1074 (37%) with subtype 1b, an 8% non significant difference ($p< 0.069$). At univariate analysis, the factors associated with SVR in patients with subtypes 1a and 1b were evaluated separately in two logistic regression models (Supplementary Table). Predictors of SVR for the two HCV subtypes at the multivariate analysis are shown in Table 2. Four factors were independently associated in subtype 1a and, according to their ranking, were IL28B polymorphism CC (OR = 5.78, CI 1.98-16.83), RVR (OR = 4.179, CI 1.66-10.55), female gender (OR = 2.83, CI 1.83-6.78), and HCVRNA ($\log_{10}$) (OR = 0.55, CI: 0.32-0.96). In patients with subtype 1b the ranking of SVR predictors was RVR (OR=6.49, CI 4.32-9.73), IL28B CC (OR=3.32, CI 2.15-4.58), γGT (OR=1.59, CI 0.14-2.22), HCVRNA (OR=0.61, CI 0.47-0.79), and age (OR=0.01, CI 0.02-0.42).

Meta-analysis of SVR rates after Peg-IFN and RIBA

Results from the considered studies are summarized in the Supplementary Table. Overall, 4467 HCV-1 infected patients were enrolled, 1286 of them (28.9%) with subtype 1a, and the remaining 3181 patients with subtype 1b. The proportion of subtype 1a patients was >50% in three studies from the USA (6-8), and <30% in most reports from European countries. Of the 8 surveyed trials, 3 studies ended up with significantly higher SVR rates after treatment with Peg-IFN and RIBA in HCV subtype 1a patients (11-13), and two studies with significantly better rates in patients with subtype 1b (8,9). By pooling results from previous studies with those of the present investigation (Figure 2), the weighted SVR rates were not different between the two viral strains: OR = 0.98, CI 0.72-1.32.

DISCUSSION

HCV genotype 1 is generally considered as a homogeneous group, but it shows genetic diversity with at least 60 members identified by nucleotide sequence variability. HCV subtypes 1a and 1b are the major strains, and show geographical differences, with subtype 1a ~~being~~ highly prevalent in the USA and subtype 1b in Europe (21,22). In accordance with the global epidemiology of HCV infection, among the studies pooled in the present meta-analysis, the prevalence of subtype 1a was >50% in the three studies from the USA (6-8), and <30% in most reports from European countries. In keeping with previous figures, the majority of our Italian patients (87%) carried the HCV subtype 1b. Up to now, genotyping for HCV-1 subtypes will not impact on the day-to-day clinical management of chronic HCV infection, whereas conflicting reports exist on their influence on the outcome following conventional therapy with Peg-IFN and RIBA (6-13). Recently, several HCV inhibitors appear to have selective activity against HCV 1 subtypes both in vitro and in vivo, with subtype 1a appearing more resistance-prone and less responsive to triple therapy than subtype 1b (25-28).

In the current study, HCV-1 subtypes profiles revealed some variation in the clinical presentation of patients infected with subtype 1a vs those infected with subtype 1b. The most differentiating characteristic was a male prevalence and a 10-year younger age of patients with subtype 1a in comparison with an equal gender distribution and older age in carriers of the subtype 1b. Of note, neither HCV RNA levels nor IL28B distribution differed in patients infected with the two subtypes. A similar age difference and male prevalence was noted in another Italian study (11). These different demographic features may have determined a less severe liver fibrosis in our patients with HCV subtype 1a, an observation in keeping with the more recent spreading of the infection with this subtype worldwide (22).

As to the sensitivity of HCV-1 subtypes to Peg-IFN and RIBA, there has been no consistency among studies in regard to a differential responsiveness of subtype 1a vs 1b on either on- and off-treatment parameters (6-13). In our investigation, HCV clearance by week 4 and 12 of treatments, and at the end of treatment did not differ between patients carrying one or the other subtype (Figure 1). However, a marginal, non-significant 8% difference in SVR rates in subtype 1a vs the other subtype was noted in our cohort, a finding which prompted us to pool our results with those available from other 8 trials addressing this topic. At the meta-analysis of results from the previous studies (Figure 2), the two viral strains were shown to respond equally well to Peg-IFN and RIBA, with a pooled OR of 0.98 (CI 0.72-1.32). This observation would signify that the different outcome between the two subtypes noted following triple therapy with Peg-IFN and RIBA in combination with a direct-acting agent (27,28) needs to be ascribed to the protease inhibitor administered, and not to Peg-IFN nor to RIBA.

The study also pinpoints to some differential predictive features of SVR between the two HCV-1 subtypes. In patients with either subtype 1a or 1b, major drivers of SVR after a full course of Peg-IFN and RIBA appeared to be the polymorphisms of IL28B and the RVR status, with patients' age and baseline viral load having a marginal influence. Of note, two other features, i.e, female sex for

subtype 1a and normal γGT levels for subtype 1b, were also shown to bear a moderate impact on a successful outcome of therapy. Fibrosis stage and prediction of viral response to treatment have been linked to γGT activity (30,), an indirect marker of oxidative stress in relation to glutathione metabolism (29). Advanced fibrosis of the liver did not correlate with SVR in our cohort, a correlation which obscured and/or incorporated by the impact of γGT on SVR. Finally, the relevance of female gender on SVR rates in patients with subtype 1a but not in those with the other subtype awaits further confirmation in a larger cohort of carriers of the former viral strain.

In conclusion, our study reveals some differential features in the presentation of patients with HCV-1 infection, with subtype 1a carriers being more frequently younger, of male gender, and with a less advanced stage of liver fibrosis. As to the claimed better efficacy of treatment with Peg-IFN and RIBA for HCV-1a patients, either the results of the present investigation as well as the pooled data from 8 other clinical trials on this topic document an equal responsiveness of the two HCV subtypes to this therapeutic regimen. IL28B genotype and attainment of the RVR status appear the major driver of a favorable outcome following Peg-IFN and RIBA therapy in both HCV-1 subtypes.

CONFLICT OF INTEREST STATEMENTS

Angelo Andriulli , Filomena Morisco, Antonio Massimo Ippolito, Vito Di Marco, Maria Rosa Valvano, Mario Angelico, Giovanna Fattovich Antonina Smedile, Rocco Granata, ' Michele Milella, Martina Felder Giovanni Battista Gaeta, Pietro Gatti, Massimo Fasano, Giuseppe Mazzella and Teresa Santantonio declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

Legend to Figures

Figure 1: Viral responses at week 4 (RVR), week 12 (EVR), end-of-treatment (EOT), and at week 24 off-treatment (SVR) in HCV-1 patients with subtype 1a or 1b.

Figure 2: Forrest plot of the studies that compared sustained viral response (SVR) rates in HCV-1 patients with subtype 1a or 1b.

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